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**Supporting information for article:**

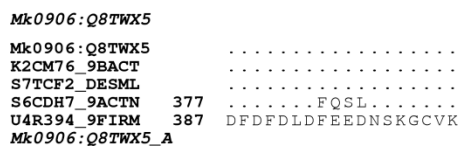
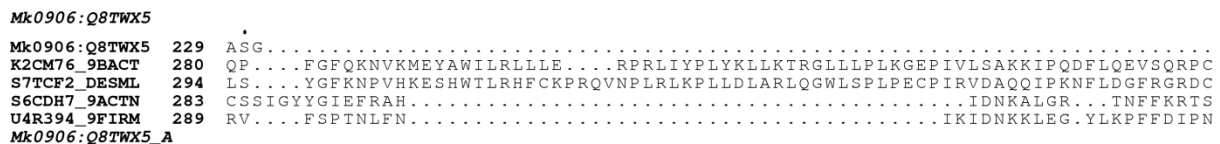
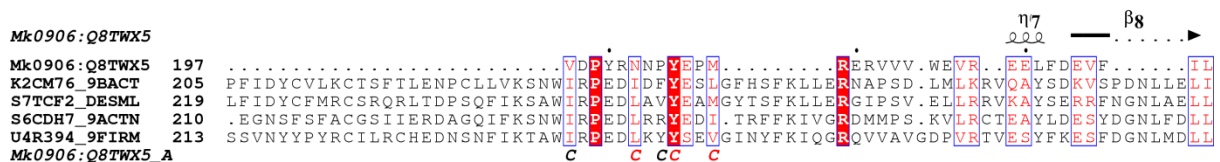
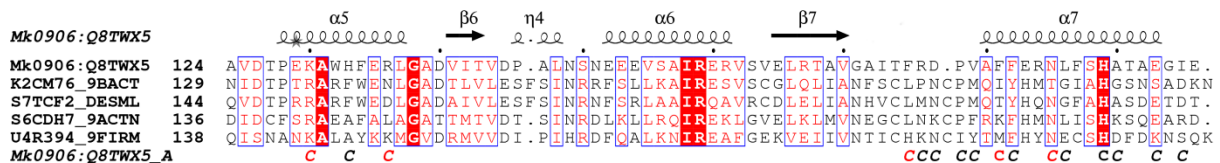
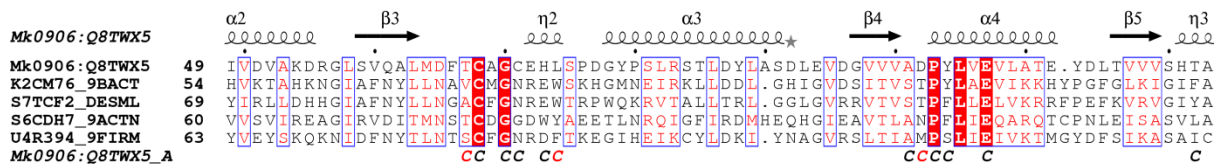
**First crystal structure of the peptidase domain of the U32 peptidase family**

**Magdalena Schacherl, Angelika A. M. Montada, Elena Brunstein and Ulrich Baumann**

## S1. Sequence of the Mk0906 construct used in this study

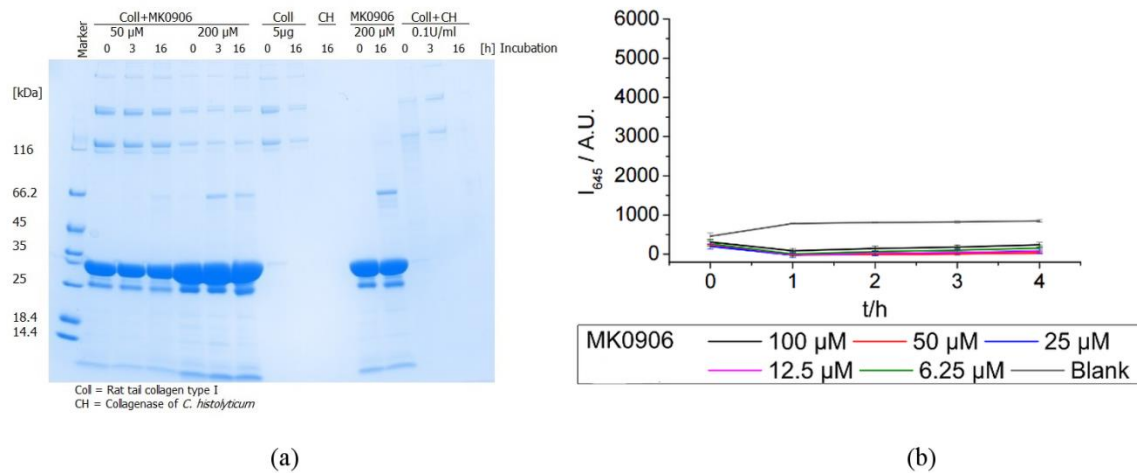
The N-terminal additional amino acids are shown in italics with the StrepII-tag being underlined and the thrombin cleavage site shown in bold letters.

*MGSWSHPQFEKSSGLVPRGSMTRRWYLCCSRHHLDTVPEDSDGIVVPVTEHGVATL*  
 LPRYPETYEVEDIVDVAKDRGLSVQALMDFTCAGCEHLSPDGYP SLRSTLDYL ASDLEVD  
 GVVVADPYLVEVLATEYDLTVVVSHTAAVDTP EKAWHFERLGADVITVDPALNSNEEEV S  
 AIRERVSVELRTAVGAITFRDPVAFFERNLFSHATAEGIEVDPYRNNPYEPMRERVVVWE  
 VREELFDEVFILASGEPP



**Figure S1** Sequence alignment of bacterial proteins exhibiting the zinc-binding sequence motifs.

K2CM76: uncharacterized protein from an uncultured bacterium; S7TCF2: U32 peptidase from *Desulfococcus multivorans*; S6CDH7: Protease from *Adlercreutzia equolifaciens*; U4R394: uncharacterized protein from *Clostridium papyrosolvens*.



**Figure S2** Proteolytic assays. (a) Collagen-digestion. 5 μg rat tail collagen (BD Biosciences, Oakpark, USA) (Mr of 115, 130 and 215 and 230 kDa) was incubated with Mk0906 (Mr 28.4 kDa) in vast excess (50 and 200 μM) for a total of 16 hours at 37°C. *C. histolyticum* collagenase (0.05U/ml) was used as positive control. Further control lanes show collagen without Mk0906 and Mk0906 alone. Lane 1: Marker (Unstained protein molecular weight marker, Fermentas); Lanes 2-4: collagen plus 50 μM Mk0906 after 0, 3, and 16 hours incubation at 37°C; lanes 5-7: collagen plus 200 μM Mk0906 after 0, 3, and 16 hours incubation at 37°C; lanes 8 and 9: collagen with buffer after 0 and 16 hours; lane 10: *Clostridium* collagenase; lanes 11 and 12: Mk0906 (200 μM) alone after 0 and 16 hours. (b) BODIPY proteolytic assay. Casein (10 μg/ml) labelled with red-fluorescent BODIPY® TR-X (E6639) dye was incubated with Mk0906 (6.25 to 100 μM) and the fluorescence was recorded for the indicated time at 37°C. Blank readings were subtracted.

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tr|Q8TWX5|Q8TWX 1 .....MTRRWYLCCSRHLDLTVPEEDSDGI.....V...VPVTEHGV
tr|Q8TZB6|Q8TZB 1 MFSVPSPGDPAALTDRL.....LRSLPDRIET.....VYFGLPIAGTGR
sp|P59916|PRTC1 .....
tr|Q9ZNF7|Q9ZNF 1 ...MAKSFEPRLVELL.....APAKDLATGQAAILHGADAVYIGAPSFGARA

tr|Q8TWX5|Q8TWX 34 ATLLPRYPETYEVEDIVDVAKDRGLSVQALMDFTCAGCEHLSPDGYPSLRSTLDYILAS
tr|Q8TZB6|Q8TZB 40 AT..PVQVDTDTATELAEVCHSYSVEPEAVINPLCTADVVC SRNAFAEFERTLDDL.D
sp|P59916|PRTC1 1 .....MRSVIDAA.Q
tr|Q9ZNF7|Q9ZNF 45 SA....GVSIEDIGRLAFCFARLYRAKVYVALNTI.....LYDDELPEAERIAWEL.Y

tr|Q8TWX5|Q8TWX 92 DLEVDGVVVDAPYLVVLAATEYDLTVVSSHATAVDTPEKAWHFELGADVITVDPALN
tr|Q8TZB6|Q8TZB 95 DAGIERLVLSDPLMIHA.AVERGFRVSVSCVVEVNTPEERARYFDEIGVEIITLDINVN
sp|P59916|PRTC1 10 KAQISAIIASDVAAMMY.ANEIGVEVHLSTQLNINSAEALRFYSRFA.DVVVLLARELN
tr|Q9ZNF7|Q9ZNF 92 RAGADALIVQDM.SLCR.LNLPPIALHASTQCDIRTVKVRMFEVLGYEQVLLARELS

tr|Q8TWX5|Q8TWX 150 SNEEVSARERV.....SVELRTAVGAI...TFRD.PVAFFERNLFSHATA
tr|Q8TZB6|Q8TZB 152 RRLDTIEATASEV.....SARLRIVNEG...CLPDCPYRASHFCLFSHATR
sp|P59916|PRTC1 66 ..MDQVRTIHEITIVRDNICGPKGHPRIEMFAHGALCMAVSGKCYLSLHEHNSANRG
tr|Q9ZNF7|Q9ZNF 148 ..LPEIRKADST.....SVLEAFVHGALCVSLSGQCYLSEALTRRSANRG

tr|Q8TWX5|Q8TWX 193 EGIEV..DPEY.....RNNPYEPMRERVV.....ELFDEVFIL.....
tr|Q8TZB6|Q8TZB 196 PGEEVAEDPEYVRCISE...RVNNPTLIKSPFVVRPEDLSVYMDLGVRAFKIAGRAN
sp|P59916|PRTC1 122 ACAQICRRGYTVKDKDSGLELDIENQYIMSPKDLKTIHFINKMMDAGVRVFKIEGRAR
tr|Q9ZNF7|Q9ZNF 193 ACAQLCRLPEYTMIDADG..KVIRSNQHLISLKDLENRSAELESMLEAGISSFKIEGRILK

tr|Q8TWX5|Q8TWX 214 ..VW.....EV.....RE.....ELFDEVFIL.....
tr|Q8TZB6|Q8TZB 250 SITWI.....RRAV.....RAYLRGRYDGNLLDILDCTVLRHLYHVDNREL.
sp|P59916|PRTC1 180 GPEYVYTVCRCYKEAI.....EAYCNGTYDEEAIGRWDE.....QLATVFNRGFW
tr|Q9ZNF7|Q9ZNF 249 GISYVKNVTAHYRRLDELISRYPERYCRASSGRCTFA.FHP.....APEKSFNRGFT

tr|Q8TWX5|Q8TWX .....
tr|Q8TZB6|Q8TZB 292 DGFL..KR.....VGRCDRR.....C.....
sp|P59916|PRTC1 225 DGYLGR.....LGEWTHRYG.....SGATRQKIYV.....GKGI
tr|Q9ZNF7|Q9ZNF 301 NLLLSGKRDTNLTITPESNKSKGAYIGCVTAVNRNKVSIRLRSNTGAKPSLANGDGL

tr|Q8TWX5|Q8TWX .....
tr|Q8TZB6|Q8TZB 306 .....SKCGFC.....
sp|P59916|PRTC1 256 KYF.....SRLGVAEFEIESEGLHIGDEI.VITG.....
tr|Q9ZNF7|Q9ZNF 359 YLLHPDGNMSGTRINVV...LPDGSIQVDNSAGIVSGTINVFRNYDIRFEKALSQDNSA

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**Figure S3** Part of the sequence alignment of Mk0906 (Q8TWX5) with *P. gingivalis* PrtC (P55916), *P. gingivalis* PrtQ (Q9ZNF7) and *M. kandleri* Mk0019 (Q8TZB6). Shown is only the N-terminal part

of the sequences of PrtC and PrtQ since the C-terminal part consists of a  $\beta$ -barrel domain similar to PDB entry 4he6 (Trillo-Muyo *et al.*, 2013). The sequence alignment was performed with MAFFT/L-INS-i (Kato *et al.*, 2005).